

SULPHUR NUTRITION IN DUCKWEED,
SPIRODELA OLIGORRHIZA

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ABSTRACT

Fronds of duckweed (*Spirodela oligorrhiza*) were grown under aseptic conditions with a variety of sulphur compounds as sole sources of sulphur. The ten sulphur compounds tested varied widely in their effects on total growth, rate of growth and appearance of the fronds.

Sulphate was used most effectively, but thiosulphate, cysteine, cystine, and cysteic acid were also utilised. Dithionate had no observable effect on growth, whilst thiourea, methionine, sulphanilamide, and sulphanilic acid all markedly depressed growth when supplied at 10^{-3} M.

It is suggested that both the form in which sulphur occurs in the molecule, and the type of substituent to which it is bound, effect the ability of a sulphur compound to be utilised for growth.

The results are discussed in terms of ecological situations in which forms of sulphur other than sulphate are available to plants.

INTRODUCTION

Sulphur is required for the nutrition of all organisms and S-containing compounds have a variety of functions in metabolism. For example, cysteine is one of the most common and biologically important amino acids since it is required for the biosynthesis of proteins and other important compounds such as Coenzyme A. Methionine is widely distributed as a protein constituent and also serves as a major source of biological methyl groups. Several coenzymes contain sulphur, namely Coenzyme A, thiamin, biotin and lipoic acid. In addition, many other S-compounds of unknown function have been identified in plants, animals and micro-organisms (Freney 1967).

Most sulphur that is available to plants occurs as sulphate ions in soil solution. This inorganic sulphate is 8 electrons deficient compared with the reduced sulphur present in organic S-compounds. Since mammals cannot reduce sulphate they are dependent on plants for their supply of reduced sulphur for synthesis of proteins, coenzymes, etc.. The biochemical mechanism by which plants incorporate inorganic sulphate into amino acids is summarised in Fig. 1.

Although plants normally use sulphate ions to supply their sulphur requirements, some plant systems can use other forms of sulphur, such as S-amino acids (Manorik 1961, Reay 1967, Formin and Astakhova 1959, Bollard 1966).

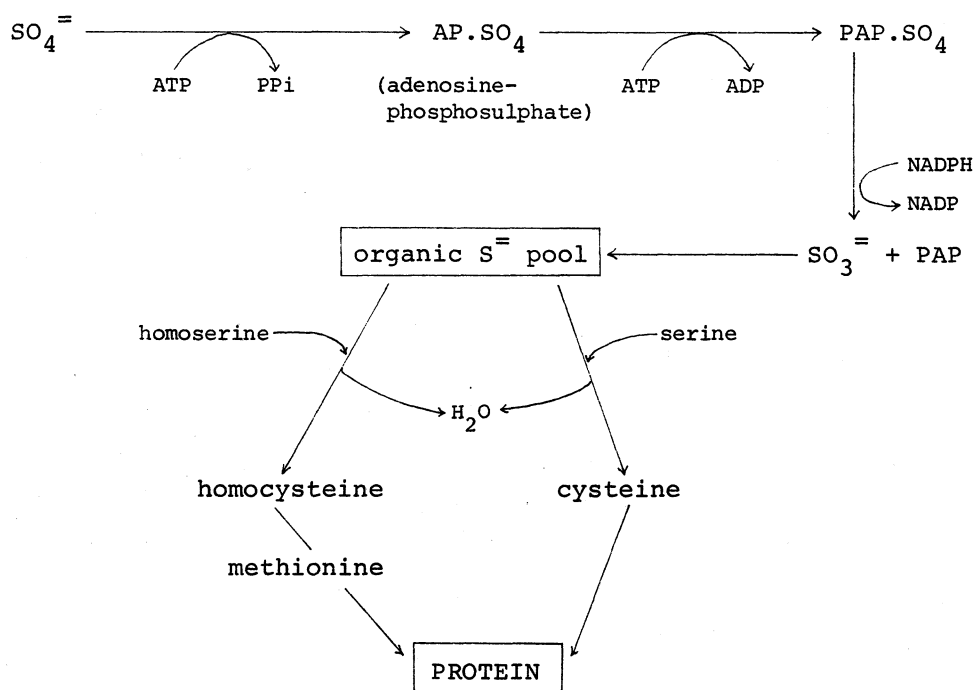


Fig.1. A scheme for the incorporation of sulphate into protein by plants (after Salisbury and Ross 1969, Krogman 1973).

The aim of this study was to test a range of S-compounds for their ability to supply the sulphur needs of a plant. The test plant was *Spirodela oligorrhiza* (duckweed), selected because it is an angiosperm that may be conveniently grown in quantity under aseptic conditions.

METHODS

GROWTH OF PLANTS

A sterile culture of *Spirodela oligorrhiza* was obtained from Dr E.G. Bollard, (D.S.I.R. Division of Plant Nutrition, Auckland, N.Z.). From this culture, an actively growing stock culture was established for use in these experiments. Each culture flask was inoculated with two naturally occurring groups of fronds (consisting usually of one juvenile and two mature fronds).

The fronds were cultured in 100 ml Erlenmeyer flasks for 22 days at 22-24°C. Light intensity was 12.92 lux; day length 16 h. The flasks were agitated daily. Each treatment was replicated 5 times and a randomised block layout of flasks in the growth room was employed to offset any effects of uneven lighting.

CULTURE MEDIA

The basal medium used was based on that of Bollard (1966). Major elements were included as:

NH_4NO_3	0.008M	KNO_3	0.001M
K_2HPO_4	0.001M	$\text{Mg}(\text{NO}_3)_2$	0.002M
$\text{Ca}(\text{NO}_3)_2$	0.002M		

Trace elements were added as below:

B	0.5 ppm	Mn	0.6 ppm
Zn	0.05 ppm	Cu	0.02 ppm
Mo	0.02 ppm	Fe	3.0 ppm
Cl	0.68 ppm		

Iron was supplied as the EDTA complex. Additional chloride was added as 2N HCl during adjustment of pH to 6.4.

Sulphur compounds were chosen to include a range in which the sulphur occurred in different forms and bound to different substituents. S-compounds that were likely to be unstable in aqueous solution during autoclaving were cold-sterilised by filtering through "Millipore" sterilising filters. Solutions of the remaining S-compounds were sterilised by autoclaving.

Cold sterilised solutions: sodium sulphite, sodium thiosulphate, sodium sulphide, thiourea, cysteine, and cysteic acid.

Autoclaved solutions: basal medium, sodium sulphate, dithionate, cystine, methionine, sulphanilic acid, and sulphanilamide.

Some S-compounds are unstable in aqueous solution, and growth on sulphide and sulphite solutions was probably due to sulphate produced by auto-oxidation (Roy and Trudinger 1970). Therefore the effects of sulphide and sulphite treatments are not discussed.

RESULTS

The sulphur compounds tested varied widely in their effects on total growth, rate of growth, and appearance of fronds of *Spirodela oligorrhiza*, as shown in Tables 1 - 3, and Figs 2 - 5.

DISCUSSION

If a S-compound is used for growth, it may be assumed that it is both assimilated and metabolised. If it has no effect on growth, the compound is either not assimilated or not metabolised. However, to cause a depression of growth, it must actually interfere with the plant's metabolism. Thus on the basis of

TABLES 1 and 2. THE EFFECTS OF S-COMPOUNDS ON THE NUMBER OF FRONDS OF *SPIRODELA OLIGORRHIZA* AFTER 14 DAYS GROWTH IN STERILE CULTURE (ORIGINAL DATA TRANSFORMED TO LOGS)

TABLE 1. ANALYSIS OF VARIANCE (WITH APRIORI INDIVIDUAL DEGREE OF FREEDOM COMPARISONS OF TREATMENT MEANS)

Source of variation	df	MS
Among treatments	12	2.636 ***
Control vs S-compounds	1	.447 **
Inorganic vs organic S-compounds	1	13.046 ***
Sulphate vs other inorganic S-compounds	1	.076 ns
Dithionate vs sulphide, sulphite and thiosulphate	1	.443 **
Among sulphide, sulphite and thiosulphate	1	.0093 ns
Amino acids vs other organic S-compounds	1	6.294 ***
Cysteine family vs methionine	1	11.220 ***
Among cysteine family	1	.0010 ns
Sulphanilic S-compounds vs thiourea	1	.365 **
Sulphanilamide	1	.461 **
Within treatments	52	.0393

Levels of significance, α

ns = not significant

** = 0.01

*** = 0.001

TABLE 2. SNK TEST BETWEEN TREATMENT MEANS
(Means that do not differ significantly from each other at the 5% level are underlined together)

Treatment:	$S_2O_6^{=}$	No sulphur	$SO_3^{=}$	$S_2O_3^{=}$	$S^{=}$	$SO_4^{=}$
Mean:	<u>8.5682</u>	<u>8.7850</u>	<u>10.0656</u>	<u>10.2946</u>	<u>10.4976</u>	<u>10.5463</u>

Treatment:	Cysteic acid	No sulphur	Cysteine	Cystine
Mean:	<u>8.5774</u>	<u>8.7850</u>	<u>9.3502</u>	<u>9.4502</u>

living fronds as an index of growth (Figs 2,3), the S-compounds can be grouped as below:

Utilised for growth: sulphate, thiosulphate, cysteine, cystine, cysteic acid.

No observable effect: no sulphur, dithionate.

Depressed growth: thiourea, methionine, sulphanilic acid, sulphanilamide.

TABLE 3. THE APPEARANCE OF FRONDS OF *SPIRODELA OLIGORRHIZA* AFTER 22 DAYS GROWTH ON VARIOUS S-COMPOUNDS AS SOLE SOURCES OF SULPHUR.

Sulphur source	Colour	Size of living mature fronds	Length of rootlet (+ +++)
No sulphur medium, dithionate	light yellow-green	large	++++
Sulphate	mid-green	large	+ to +++
Sulphide, thiosulphate, sulphite	mid-green	large	+
Thiourea, methionine	white	small	+
Cysteine, cystine	dark green	medium	+
Cysteic acid	pale green	small	++ to +++
Sulphanilic acid	white	small	+
Sulphanilamide	white or pale green	small	+

Chlorophyll concentration in mature fronds was also a good indicator of whether a compound was utilised, toxic, or without observable effect (Table 3). Application of utilisable compounds allowed continued chlorophyll synthesis, while toxic compounds led to chlorophyll degradation. No sulphur and dithionate resulted in reduced chlorophyll synthesis.

In addition to a characteristic light yellow-green colour of the fronds, sulphur starvation also led to the production of very long roots (Table 3).

As expected, sulphate was the most efficiently used sulphur source in terms of overall growth and rate of growth of the fronds. However, *Spirodela* was also able to utilise thiosulphate and the amino acid, cystine, nearly as efficiently as sulphate. Moreover, the fronds initially used cystine more efficiently than sulphate (Fig. 3).

Fronds grown on cysteine, cystine and cysteic acid gave rate-time curves of different shapes (Figs. 4,5). The growth rate of fronds grown on cysteine was remarkably constant over a ten day period. Initially, cysteine was used more efficiently than cysteic acid, i.e. the plant appeared to utilise a molecule with a thiol group (-SH) more rapidly than the equivalent molecule with a sulphonic acid group (-SO₃H).

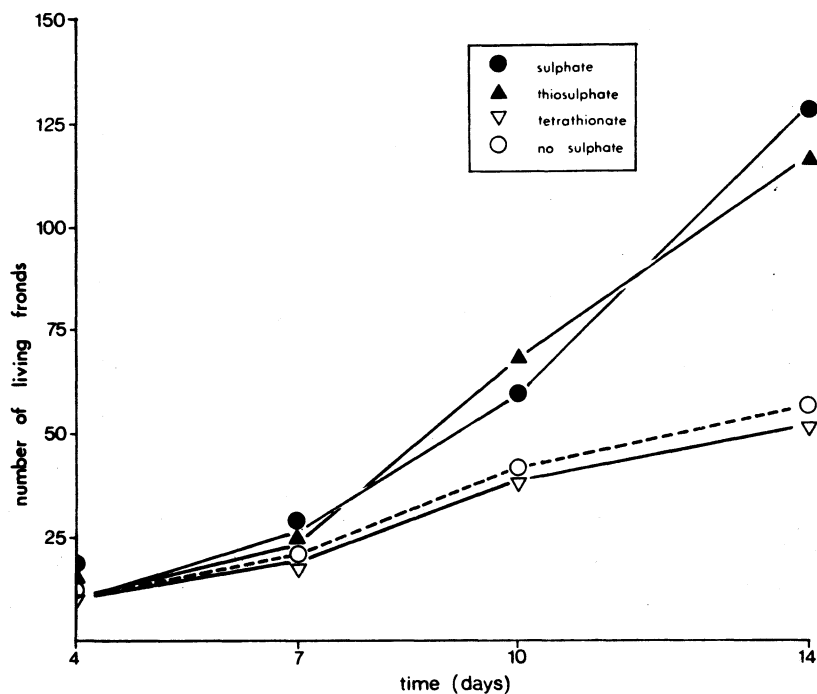


Fig. 2. Growth of *Spirodela*, measured as increase in numbers of living fronds, on inorganic sulphur compounds as the sole source of sulphur.

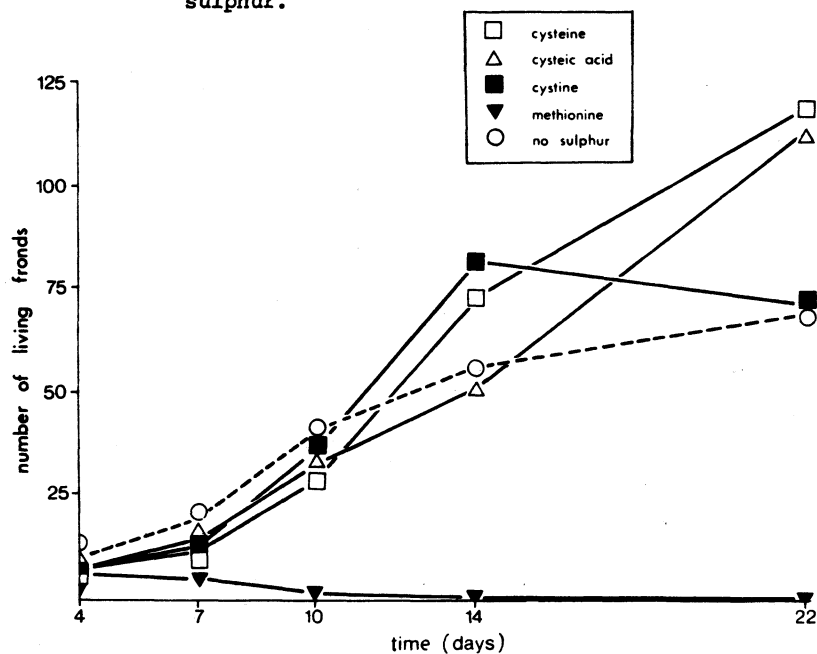


Fig. 3. Growth of *Spirodela*, measured as increase in numbers of living fronds, on sulphur amino acids as the sole source of sulphur.

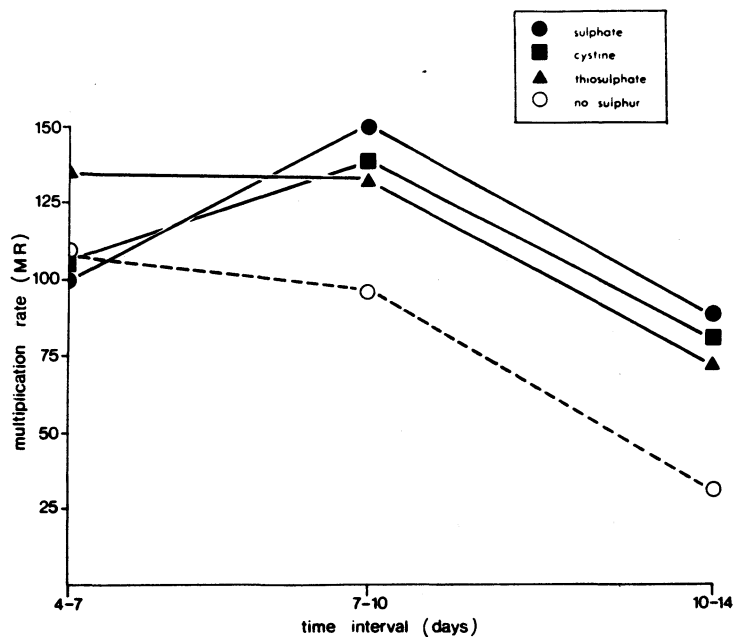


Fig.4. Rate of growth of *Spirodela* on sulphate, thiosulphate and cystine.

$$MR = \frac{\log_{10}(F_n F_o) (1000)}{n}$$

where F_o and F_n are initial and final numbers of fronds respectively, and n is the number of days over which growth rate is determined

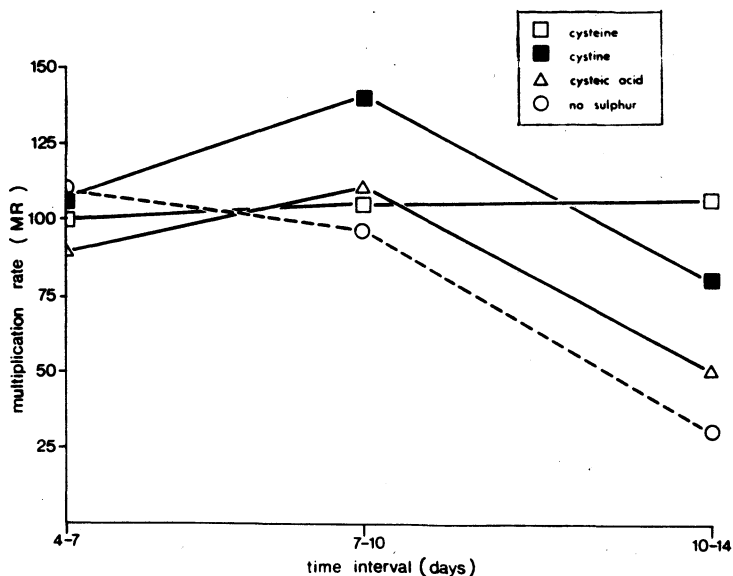


Fig.5. Rate of growth of *Spirodela* on cysteine, cystine and cysteic acid.

THE SIGNIFICANCE OF THE STRUCTURE OF THE SULPHUR COMPOUNDS

Forms of sulphur in the molecule

The compounds with sulphur occurring as sulphonic acid ($-\text{SO}_3\text{H}$), thiol ($-\text{SH}$) and disulphide ($-\text{S}-\text{S}-$) groups (i.e., cysteic acid, cysteine, and cystine) were utilised for growth by *Spirodela* (Table 4). By contrast, the compounds with thioether ($\text{C}-\text{S}-\text{C}$) and thiocarbonyl ($\text{C}=\text{S}$) structures (methionine and thiourea) were not utilised. Thus the form in which sulphur occurs in the molecule appears to affect its ability to be used for growth.

TABLE 4. STRUCTURE OF SULPHUR AMINO ACIDS AND THIOUREA

cysteic acid	$\text{HO}_3\text{S}.\text{CH}_2.\text{CHNH}_2\text{COOH}$
cysteine	$\text{HS}.\text{CH}_2.\text{CHNH}_2\text{COOH}$
cystine	$\begin{array}{c} \text{S}.\text{CH}_2.\text{CHNH}_2\text{COOH} \\ \\ \text{S}.\text{CH}_2.\text{CHNH}_2\text{COOH} \end{array}$
methionine	$\text{H}_3\text{C}.\text{S}.\text{CH}_2\text{CH}_2.\text{CHNH}_2\text{COOH}$
thiourea	$\begin{array}{c} \text{H}_2\text{N}.\text{C}.\text{NH}_2 \\ \\ \text{S} \end{array}$

The toxic effect of methionine was unexpected, since it is widely distributed as a protein constituent. Bollard (1966) also reported that methionine inhibited growth of *Spirodela* in sterile culture when supplied at 59 ppm. However, it has been shown that methionine can be used by some higher plants, e.g., rice and mustard (Formin and Astakhova 1959), sugar beet roots (Manorik 1961), and excised tomato roots when it is supplied at low concentrations (Reay 1967). Moreover, Manorik (1961) claimed that sugar beet roots preferred methionine and cysteine to sulphate as a source of sulphur.

The effect of phenyl substituents

The sulphur in both cysteic and sulphanilic acids occurs as the sulphonic acid group (Table 5). Cysteic acid was utilised, but the phenyl containing compounds sulphanilic acid and sulphanilamide were toxic.

TABLE 5. STRUCTURES OF CYSTEIC ACID, SULPHANILAMIDE, SULPHANILIC ACID

cysteic acid	$\text{HOOCNH}_2\text{CH}_2-\text{SO}_3\text{H}$
sulphanilic acid	$\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{SO}_3\text{H}$
sulphanilamide	$\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{SO}_2\text{NH}_2$

However, further investigation is needed to establish more fully the range of structures of S-compounds that can be utilised, in particular, whether aromatic S-compounds and S-compounds with thioether and thiocarbonyl structures can be utilised.

ECOLOGICAL ASPECTS

Plants are thought to derive most, if not all, of their sulphur requirements from sulphate ions. Canterbury rivers typically contain 3-20 mg of sulphate/litre (i.e., up to one-fifth of the concentrations supplied in these experiments).

The results of this study and of other workers (previously cited) demonstrate that forms of sulphur other than sulphate can be used. Therefore, it is relevant to consider ecological situations in which such forms of sulphur occur in significant amounts. These include water-logged soils (where sulphides of iron, nickel, and copper are the main forms of sulphur available to plants), and sections of rivers below outfalls from various industrial complexes, e.g., 8-65 mg sulphide/litre has been observed below an abattoir and fell-mongery (North Canterbury Catchment Board, personal communication). Sewage oxidation ponds contain 0.01 mg sulphide/litre in the water and much higher concentrations in the sludge (Christchurch Drainage Board, personal communication). How do plants in these situations obtain their sulphur? Do they utilise organic S-compounds directly or do they rely on micro-organisms to transform them into inorganic sulphate?

In addition, soils and various eutrophic situations contain appreciable quantities of organic S-compounds. Moreover, according to Freney (1967), little is known about the types and amounts of organic S-compounds that occur in the soil. Although most of the sulphur in top-soils in humid regions is in the organic form, we know little of the nature of the sulphur-containing organic matter in soil, and the mechanism of its formation, stabilisation and decomposition. Without this knowledge we cannot properly assess the contribution that organic S-compounds may make to plant growth.

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